The role of the histaminergic system on the inhibitory effect of ghrelin on feed intake in broiler chickens

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Summary

This study was conducted to investigate the possible involvement of the histaminergic system in the mediation of ghrelin-induced feeding behavior in broiler chickens. In the trial 1, the effect of intracerebroventricular (ICV) injection of ghrelin on feed intake was examined in 3-h feed-deprived broiler chickens. In the trials 2 and 3, the chickens were received ICV chlorpheniramine (an H_1 receptor antagonist) and cimetidine (an H_2 receptor antagonist) prior to ghrelin injection, respectively. Cumulative feed intake was measured at 3-h post injections. Infusion of ghrelin (0.3, 0.6 and 1.2 nmol) decreased feed intake dose-dependently ($P<0.05$). Pre-treatment of chlorpheniramine (100 μg) attenuated the inhibitory effect of ghrelin on feed intake ($P<0.05$), while such an effect was not noticed by pre-injection of cimetidine (100 μg). The results suggest that there is an interaction between ghrelin and the histaminergic system (through H_1 receptors) on control of feed intake in broiler chickens.

Key words: Ghrelin, Histaminergic system, Feed intake, Chicken

Introduction

Ghrelin, a 28-amino acid residues polypeptide, has been isolated from the rat stomach as the endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R) (Kojima *et al*., 1999). The gastrointestinal tract is recognized as the main site for ghrelin production. The highest expression of the ghrelin gene has been found in the mucosal layer of stomach fundus (Kojima and Kangawa, 2005). Ghrelin is a potent stimulus for the release of growth hormone from anterior pituitary cells in mammals. Besides GH-releasing activity, it stimulates feed intake and increases fat stores in mammals, either when administered peripherally or intracerebroventricularly (ICV) (Tschöp *et al*., 2000; Nakazato *et al*., 2001; Date *et al*., 2002). The GHS-R mRNA is widely distributed in several parts of the brain such as the hypothalamus, telencephalon, etc. (Geelissen *et al*., 2003), supporting the existence of interactions between ghrelin and neuromodulators in control of feeding behavior. In the case of mammals, the orexigenic activity of ghrelin is mediated by some neuropeptides such as neuropeptide-Y (NPY), agouti-related protein (AGRP) and orexin (Nakazato *et al*., 2001; Toshinai *et al*., 2003).

Ghrelin has also been isolated from chickens. This form consists of 26 amino acids. In chicken, ghrelin mRNA expression is detected in the highest level in the proventriculus (Kaiya *et al*., 2002). It has been shown that an intravenous (IV) injection of ghrelin transiently increases plasma GH levels (Kaiya *et al*., 2007), however ICV or IV administration of ghrelin or other growth hormone secretagogues (GHS) like GHRP-2 strongly inhibits feed intake in a dose-dependent manner in chickens (Furuse *et al*., 2001; Saito *et al*., 2002; Saito *et al*., 2005; Khan *et al*., 2006).

This effect is opposite to that of mammals. The mechanism underlying the ghrelin-induced hypophagia is still unclear, although it has been reported that an anorexic effect of ghrelin is mediated by corticotropin-releasing factor (CRF), but not NPY (Saito *et al*., 2005).

The hypothalamus is recognized as a crucial interface between afferent peripheral signals, CNS wiring, and efferent neuroendocrine axis regulating energy balance in concert (Kojima and Kangawa, 2005). Histaminergic neurons in the brain are localized in the posterior hypothalamus, which is collectively named the tuberomammillary nucleus (TMN) and projects axons to satiety centers (Watanabe *et al*., 1984; Onodera *et al*., 1994). The hypothalamic neuronal histamine is suggested to be involved in the regulation of feed intake. For example, chronic infusion of histamine into the suprachiasmatic nucleus suppresses feed intake in the rat (Itowi *et al*., 1988). On the other hand, both central (Tuomisto *et al*., 1994) and peripheral (Vaziri *et al*., 1997) infusions of α-fluoromethylhistidine (FMH), a specific and irreversible inhibitor of histidine decarboxylase, increases feed intake and feeding-associated locomotor activity (Sakai *et al*., 1995). In chickens, like mammals, neuronal histamine acts to inhibit eating so that ICV administration of histamine or increase of endogenous histamine by injection of thioperamide, a selective antagonist of histamine H_3 receptors, inhibits feed intake in chickens (Meade and Denbow, 2001; Taati *et al*., 2009). The signal inputs to the TMN may influence histaminergic function. It has been reported that the expression of the leptin-induced anorectic effect is mediated by the histaminergic system through histamine H_1 receptors (Morimoto *et al*., 1999), and leptin increases histamine release from the rat hypothalamus (Morimoto *et al*., 2000), thus, the histaminergic system likely participates in the control of feed intake down stream of other feeding related peptides.

It has been revealed that after ICV injection of ghrelin, there was an increase in c-fos expression in the hypothalamus including the TMN, suggesting the activation of histaminergic neurons (Nakazato *et al*., 2001). Furthermore, Guan *et al*. (1997) observed that the TMN expresses mRNA of GHS-R, to which ghrelin is an endogenous ligand. These reports suggest that histaminergic neurons may respond to ghrelin. Since ghrelin and neuronal histamine have the same effect on feeding behavior in chickens, the endocrine link between the digestive tract and the central nervous system in this regard is possible. Thus, we hypothesized that the central histaminergic system is a target of ghrelin in its control of feed intake, and examined the effects of blocking H_1 and H_2 histamine receptors on ghrelin-induced feeding behavior in broiler chickens.

Materials and Methods

Animals and drugs

One-day-old male Ross broiler chicks were provided from Dorbar Hatchery (Borojerd, Iran). The birds were kept in a temperature-controlled room at 33°C under continuous lightening regimen, and had free access to water and a commercial diet (22% crude protein and 3100 kcal/kg metabolizable energy, Omidi Poultry Breeding Center, Khorramabad). Cimetidine and chlorpheniramine maleate were supplied by Sigma Aldrich Co. (St. Louis, USA). Rat ghrelin was purchased from Tocris Cookson Co. (Bristol, UK). All drugs were dissolved in physiological saline to concentrations that allowed delivery of the appropriate dose in 10 µl and 5 µl injections per chicken by using a microsyringe. The trial was performed in accordance with the Animal Care Committee of Lorestan University, School of Veterinary Medicine.

Surgical preparation

At the third week of age and weights of 750 g, the birds were anaesthetized intramuscularly with ketamine (30 mg/kg) and xylazine (1 mg/kg) (Thurmon *et al*., 1996) and then placed on a stereotaxic apparatus (Pooyan, Mashhad, Iran). For the ICV injection, a stainless steel guide cannula (23-gauge) was implanted into the lateral cerebral ventricle according to Davis *et al*. (1979). The stereotaxic coordinates for the guide cannula were 6.7 mm anterior to bregma, 0.7 mm lateral to the midline, and

3.7 mm below the outer surface of the skull. The guide cannula was fixed to the skull with three stainless steel screws and dental cement (Aqua Cem, Dentsply) on the dorsal surface of the skull. A stainless wire stylet was placed in the guide cannula. Lincospectin (Razak) was applied to the incision to prevent infection. The birds were allowed a minimum of 5 days recovery prior to injection.

Experimental procedure

Three trials were conducted to determine the possible interaction between ghrelin and neuronal histaminergic system on the feed intake of chickens. In each, eight birds were used in a replicated 4 × 4 Latin square design in which birds and days were the blocking factors. So that, in all trials eight chickens were arranged in four groups and each group per day received one of the A, B, C, or D treatments (for example, a combination of chlorpheniramine with ghrelin and saline in trial 2, Table 1). All solutions were injected at 2-day intervals so that each group received each solution during a 7-day test period. Injections were made with a 29-gauge, thin-walled stainless steel injection cannula which extends 1 mm beyond the guide cannula. This injection cannula was connected to a 10 µl Hamilton syringe via a 30 cm length of polyethylene (PE) tubing. Solutions were injected over a 30 sec period and the injection cannula remained in place for an additional 30 sec before removal. All injections were made at 1100 h daily, and the birds were returned to their cages after injection. Proper location of the guide cannula was verified by intracerebroventricular injection of methylene blue and anatomically slicing the frozen brain tissue at the end of the trials.

In trial 1, after being deprived of feed for 3 h, the birds were infused ICV with 0, 0.3, 0.6 and 1.2 nmol of ghrelin in a volume of 10 µl into the right lateral ventricle. Cumulative feed intake was measured at 15, 30, 60, 120 and 180 min after injection. The weight of feeders was measured using an electric digital balance of precision ± 1 g. This trial was performed to determine the effective and appropriate dose of ghrelin on the feed intake of fasted chickens. Furthermore, the results from trial 1 were used to determine the submaximal dose of gherlin which it was applied for the injections in the next trials.

In trial 2, the chickens received 100 µg chlorpheniramine before infusion of 0.6 nmol ghrelin. Each bird was given two injections of 5 µl in 5 min time interval as described in Table 1. Feed intake (g) was measured as in trial 1. The dose of chlorpheniramine was determined from our preliminary study (Taati *et al*., 2009). Trial 3 was similar to trial 2 except that the chickens received ICV cimetidine (100 µg) followed by ghrelin (0.6 nmol) 5 min later. In all trials, normal saline was used in control groups.

Table 1: Procedure of receiving treatments by chickens in trial 2 with a replicated Latin square design (day 1)

Groups	Treatments	Injections
I (n = 2)	A	Saline+saline
II (n = 2)	B	Saline+ghrelin
III (n = 2)	C	Chlorpheniramine+saline
VI (n = 2)	D	Chlorpheniramine+ghrelin

Statistical analysis

All results are given as mean ± SEM. The statistical differences between the values of the mean cumulative feed intake (g) at each time period were analyzed using a one-way ANOVA. Comparisons between means were carried out using the Tukey test as a post hoc. A p-value less than 0.05 was considered statistically significant.

Results

In trial 1, the ICV infusion of various doses of ghrelin caused a dose-dependent and statistically significant decrease in feed intake from 15 to 60 min after injection ($P<0.05$, Fig. 1) in which the dose of 1.2 nmol was more effective than other doses. Cumulative feed intake with ICV injection of 0, 0.3, 0.6 and 1.2 nmol of ghrelin at 60 min after injection was 14.57, 7.85, 6.14 and 4.85 (g), respectively. Ghrelin at a dose of 0.3 nmol did not have a significant effect on feed intake within the second and third h after injection (17 g and 25.57 g, respectively), while at doses of 0.6 and 1.2 nmol it significantly influenced feed

consumption during the last 2 h of the trial (Fig. 1). In trial 2, injection of chlorpheniramine with saline significantly ($P<0.05$, Fig. 2) increased feed intake during the 2 h after the beginning of ICV infusion as compared with the control group (24 g versus 18.5 g, respectively). The decrease in feed consumption induced by ghrelin was significantly attenuated by pre-injection of 100 µg chlorpheniramine and returned to control level only for 30 min after the beginning of the injection in this trial, but not at later points. Intracerebroventricular injection of 100 µg cimetidine had no significant effect on feeding when infused with saline in trial 3 (Fig. 3). In this trial of our study, although ghrelin at a dose of 0.6 nmol significantly decreased feed intake in comparison with the control group in all periods after infusion, pre-injection of

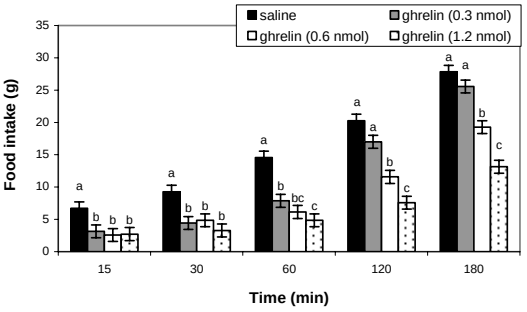

Fig. 1: Cumulative feed intake in the control and ghrelin (0.3, 0.6 and 1.2 nmol) - treated chickens at different times after injection. Values are means ± SEM. Different letters (a, b and c) indicate significant differences between treatments ($P<0.05$)

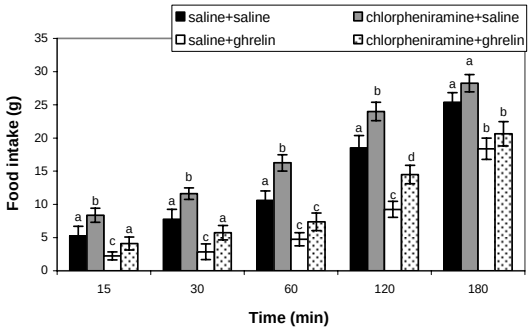

Fig. 2: Cumulative feed intake in the control and chlorpheniramine (100 µg) + ghrelin (0.6 nmol) - treated chickens at different times after injection. Values are means ± SEM. Different letters (a, b, c and d) indicate significant differences between treatments ($P<0.05$)

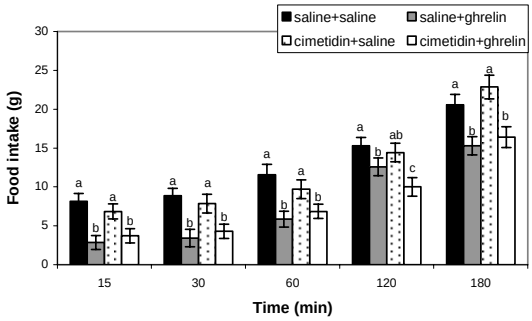

Fig. 3: Cumulative feed intake in the control and cimetidine (100 µg) + ghrelin (0.6 nmol) – treated chickens at different times after injection. Values are means ± SEM. Different letters (a, b and c) indicate significant differences between treatments ($P<0.05$)

cimetidine had no significant effect on ghrelin-induced suppression of feed intake.

Discussion

It is a well known physiological fact that ghrelin acts as an orexigenic (appetite inhancer) peptide in mammals when injected centrally and peripherally (Tschöp *et al*., 2000; Nakazato *et al*., 2001; Date *et al*., 2002). Conversely, our results in the first trial of this study which showed that ICV injection of rat ghrelin inhibits dose dependent feeding behavior in broiler chickens (Fig. 1), which is in accordance with other reports in birds (Furuse *et al*., 2001; Saito *et al*., 2002; Shousha *et al*., 2005). This trial was conducted to achieve the proportion and effective dose of ghrelin on feed intake. Rat ghrelin was used in this investigation. Since the amino acid sequence of rat ghrelin is quite different from that of the chicken hormone, except for the seven N-terminal residues (Kaiya *et al*., 2002), rat ghrelin could possibly act as an antagonist of the chicken GHS-R, thus inhibiting the effects of endogenous chicken ghrelin. However, this inhibition might not be due to an antagonistic effect of rat ghrelin at the chicken ghrelin receptor, since ICV injections of chicken ghrelin also inhibited feed intake in chickens (Saito *et al*., 2002). Although, central injection of rat or chicken ghrelin always inhibits feed intake in birds (Furuse *et al*., 2001; Saito *et al*., 2002), intraperitoneal administration of ghrelin increased feed intake in Japanese quail at

low doses, but high doses of ghrelin caused hypophagia (Shousha *et al*., 2005), therefore, it seems that the mechanisms underlying the central and peripheral effects of ghrelin on feeding in chickens are different. A low amount of ghrelin can reach central GHS-R through the blood-brain barrier and inhibit feed intake when injected intraperitonealy (Shousha *et al*., 2005). Peripheral ghrelin acts via gastric vagal afferent nerves (Date *et al*., 2002). The ghrelin signals from peripheral GHS-R may be converted to neurotransmitter-mediated signals in the nucleus of the solitary tract. This neurotransmitter could have the opposite effect to direct release of ghrelin in the hypothalamus (Shousha *et al*., 2005). Thus, the increase of plasma ghrelin levels as seen naturally after fasting in chickens (Richards *et al*., 2006; Kaiya *et al*., 2007) as well as rats (Tschöp *et al*., 2000; Toshinai *et al*., 2001), acts as a hunger signal rather than a satiety signal.

In the next trials (2 and 3) of the present study, H_1 and H_2 receptors were blocked by chlorpheniramine and cimetidine, respectively. Intracerebroventricular infusion of chlorpheniramine as an antagonist of H_1-receptors enhanced the feed intake. This result is consistent with other reports that demonstrate endogenous histamine in the brain exerts an inhibitory effect on feeding behavior through H_1-receptors in mammals (Fukagawa *et al*., 1989; Doi *et al*., 1994; Sakata *et al*., 1997) and chickens (Meade and Denbow, 2001; Taati *et al*., 2009), however, ICV injection of cimetidine, a H_2-receptor antagonist, had no effect on appetite, which is in line with the earlier findings (Sakata *et al*., 1988; Ookuma *et al*., 1989; Mercer *et al*., 1994; Lecklin *et al*., 1998; Taati *et al*., 2009). Thus, it may be concluded that brain histamine inhibits feed intake via H_1 but not H_2-receptors in chickens as well as mammals. For evaluation of possible interaction between the ghrelin and histaminergic system in feeding behavior, we used ghrelin following the blockade of H_1 and H_2 receptors, respectively. It was found that ICV pre-injection of chlorpheniramine, attenuated the inhibiting effects of ghrelin on feed intake and returned feed consumption to control levels by 30 min after the beginning of the trial, whereas cimetidine had no significant effects on ghrelin-induced suppression of feed intake. On the basis of our data, it is possible to suggest that the histaminergic system could be one of the targets of ghrelin in control of appetite in chickens. However, a recent study showed that ghrelin expresses its action in a histamine-independent manner in mammals. Ishizuka *et al*. (2006) revealed that ghrelin does not affect histamine release from histaminergic neurons and it increases feed intake, even in H_1-receptor knockout (H1R-KO) mice. In fact, it has been proved that there are differences in the orexigenic pathway of the central nervous system among birds and mammals (Shousha *et al*., 2005). For instance, ICV injection of orexin increases feed intake in mammals, whereas this orexigenic peptide has no effect on feeding behavior of birds (Furuse *et al*., 1999). Thus, it is possible that ghrelin have an opposite effect on the histaminergic system in birds.

Recently, Saito *et al*. (2005) demonstrated that the inhibitory effect of central ghrelin on feed intake in chickens is caused by activating the CRF (corticotropin-releasing factor) system, so that co-injection of a CRF receptor antagonist, astressin, attenuated ghrelin-induced increase in plasma corticostron and anorexia in neonatal chicks (Saito *et al*., 2005). It has been shown that central administration of histamine or its agonists leads to increased levels of CRF and corticostron in pituitary portal blood and peripheral plasma, respectively (Kjaer *et al*., 1991, 1993). Likewise, histaminergic neurons originating in the posterior hypothalamus project to the paraventricular nucleus and the supraoptic nucleus (Inagaki *et al*., 1988; Panula *et al*., 1989) where CRF neurons are located (Swanson *et al*., 1983). Taken together it is concluded that ghrelin acts on CRF release, likely through activation of the histaminergic system and in turn decreases the feed intake in chickens. Since pre-injection of chlorpheniramine attenuated the suppressive effect of ghrelin on appetite only within 30 min after the beginning of the injection and feed consumption has not increased during the next period of the trial, an assumption might be made; activation of the histaminergic

system by ghrelin tends to inhibit feed intake in satiety centers through H_1 receptors, and at the same time tends to increase CRF secretion. The latter increased function of the CRF system and the plasma level of corticostron has a prolonged inhibitory effect on feed intake.

In conclusion, our data in this study shows that ghrelin can directly affect the histaminergic system. Nevertheless, we can not rule out the interaction between histaminergic and CRF systems in ghrelin-induced inhibition of feed intake in broiler chickens. Further investigation is needed to clear the possible pathways by which ghrelin affects feeding behavior in broiler chickens.

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